APPLICATION NOTE

Bacterial Cell Disruption using Bioruptor® Plus

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Introduction

Disruption of bacterial cells is required for releasing biological molecules from within the cells for various biological applications. Ultrasound technology has been widely used for such cell lysis applications. Ultrasonic disruption is based on cavitation, the creation of cavities in cell suspension. These cavities or small bubbles of dissolved gases or vapors arise from the alteration of pressure in liquids. The bubbles are capable of resonance vibration and produce vigorous eddying or microstreaming leading to mechanical stress that effectively lyses cells. Diagenode's Bioruptor® sonicaton device uses state-of-the-art ultrasound technology to disrupt cells for biological, chemical, pharmaceutical, and industrial applications.

Bioruptor® offers unique benefits for cell lysis:

- Simple
- Rapid
- No contamination between samples
- Efficient
- Gentle processing
- Reproducible
- Multiplexing capability

We describe here a simple, effective and rapid protocol for lysis of bacteria using the Bioruptor®.

Methods

Culture conditions:

Recombinant *E. coli* strain (BL21) along with pGEM expression plasmid coding for GST protein were used in the study. Initial bacterial inoculum was prepared in a shake flask culture and grown overnight at 37°C with shaking. It was then transferred to 200 ml of fresh medium (ImMedia Amp liquid, Invitrogen) and grown to OD_{600} 0.6-0.7. GST was induced by IPTG (Sigma) to a final concentration 1 mM. The induction of GST protein was confirmed by SDS-PAGE and only bacterial cultures with expressed GST protein were used for the assays described here. Cells were collected by centrifuging for 15 minutes, washing twice with cold PBS, and resuspending in PBS containing protease inhibitors cocktail to OD_{600} 3.0.

Bacterial cells disruption by sonication with Bioruptor®:

Cell suspensions were transferred into Diagenode's 1.5 ml TPX tubes (M-50001) or 10 ml tubes (AS-100). Sample volumes were 300 µl for 1.5 ml TPX tubes and 2 ml for 10 ml tubes. Samples were sonicated with the Bioruptor® Plus (UCD-300) at High Power for times ranging from 5 to 20 minutes. Diagenode's Water Cooler (BioAcc-cool) was used to maintain the temperature at 4°C. An aliquot of each of the sonicated lysates was used for quantification of the cell disruption. The remaining sonicated samples were centrifuged at 14,000 rpm for 5 minutes (4°C) to pellet debris. Supernatants were used for analysis.

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Cell disruption quantification:

The number of intact remaining cells after sonication was estimated by OD_{600} and by the Live/Dead BacLight Bacterial Viability Kit (Molecular Probes).

Protein quantification and SDS-PAGE:

The soluble proteins released after sonication were quantified using the Qubit Protein Assay Kit (Invitrogen). An aliquot of each sample was analyzed in 10% SDS-PAGE stained with Coomassie Blue (Cosmo Bio, Japan).

GST activity measurement:

GST activity was determined in the supernatant of sonicated samples using the GST Activity Kit (Sigma). The activity was normalized to the protein amount in the sample.

DNA quantification and agarose gel:

DNA release was quantified using the Quant-iT dsDNA BR Assay Kit (Invitrogen). An aliquot of sonicated samples was analysed in a 1.5% agarose gel stained with SYBR Safe (Invitrogen).

Results

Efficient cell disruption with Bioruptor®

Cell suspensions were sonicated for different periods of time ranging from 5 to 20 minutes. Two types of tubes were tested: Diagenode's 1.5 ml TPX tubes (M-50001) and Diagenode's 10 ml tubes (AS-100). The efficiency of cell disruption was initially determined by measuring optical density at 600 nm. The results indicated that the number of intact cells decreases rapidly with increasing sonication time. After only 5 minutes of sonication, a significant number of cells were disrupted (Fig.1). Similar results were observed using the Live/Dead BacLight kit (data not shown) which allows the quantification of live cells with intact membrane and discrimination from cells with damaged membranes. Thus, efficient cell disruption is observed after 5-10 minutes of sonication.

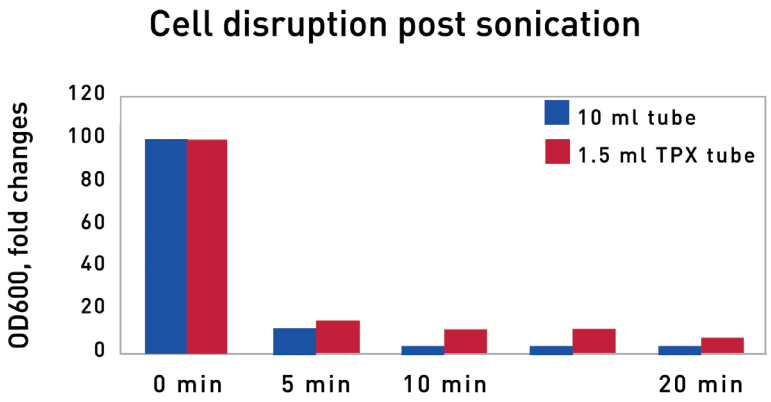

Figure 1: Effect of sonication on cell disruption
The number of intact cells after sonication was measured by optical density at 600 nm. Optical density of cell culture before sonication (0 min) is arbitrarily set to 100%.

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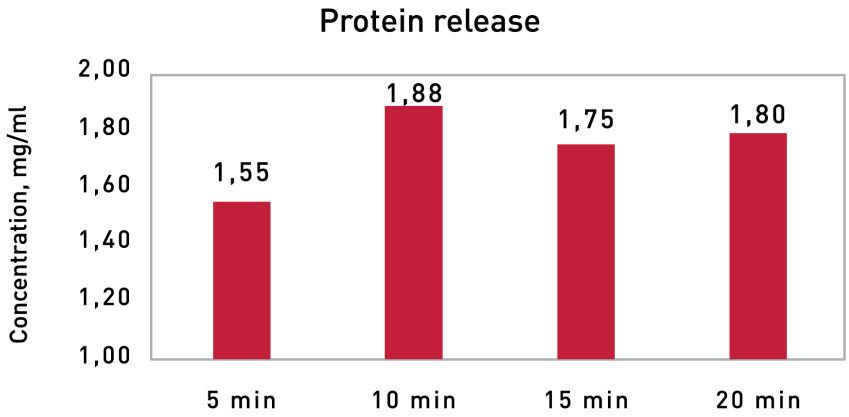
Soluble protein release correlates with cell disruption

Following cell disruption by sonication, the soluble fraction was separated from the cell debris by centrifugation. The soluble proteins released after sonication in the 10 ml Diagenode tubes were quantified (Fig. 2, A) and analyzed by SDS-PAGE. The results show that protein release correlates with cell disruption. The maximum protein recovery occurs after 10 minutes of sonication. Sonication in 1.5 ml tubes leads to the similar values (data not shown).

A.



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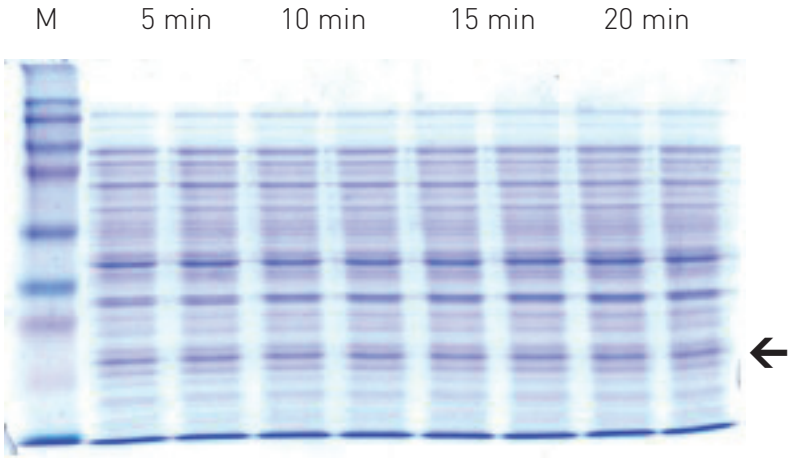

Figure 2: Effect of sonication on protein release

Figure A: The amount of protein after each sonication time was quantified by using the Qubit Protein Assay Kit (Invitrogen).

Figure B: An aliquot of sample duplicates of released protein after each sonication time was analyzed by SDS-PAGE. Lane M represents the molecular weight marker. The arrow shows expressed GST protein (checked by Western blot, data not shown).

Specific enzymatic activity remains after sonication

We further investigated the effect of sonication on enzymatic activity of recombinant GST. Specific activity remains at the same level after 5-10 minutes of sonication (Fig.3). Longer sonication leads to a decrease of GST activity in the samples.

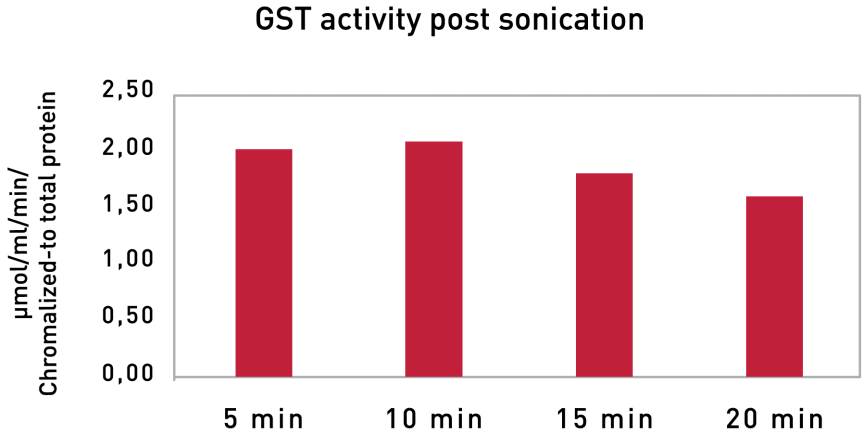

Figure 3: Effect of sonication on GST enzymatic activity

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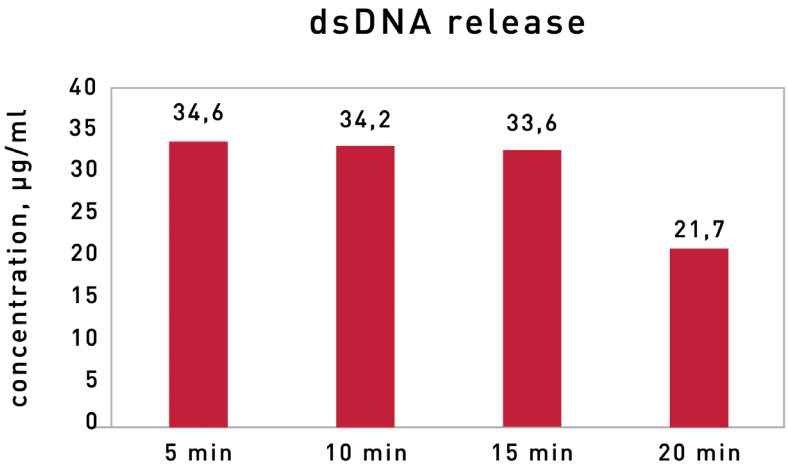
Sheared DNA is released during bacterial sonication

The disruption of bacterial cells by sonication releases DNA with maximum recovery after only 5 minutes of treatment (Fig. 4, A). The released DNA is fragmented with fragment size dependent on sonication time (Fig.4, B).

A.



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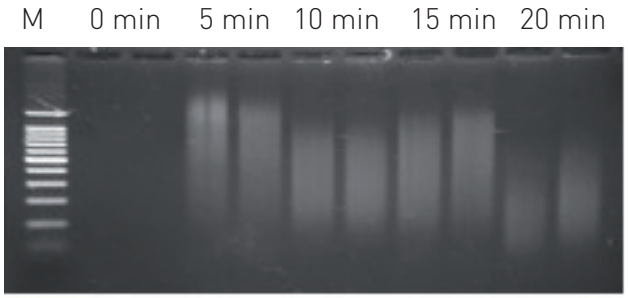

Figure 4: Effect of sonication on DNA release

Figure A: The DNA concentration in each sample after sonication was quantified with the DNA BR Assay Kit (Invitrogen)

Figure B: An aliquot of each sample, before (0 min) and after sonication was run on a 1.5% agarose gel stained with SybrSafe and visualized in UV light. Lane M represents a 100 bp ladder.

Conclusions

Efficient cells disruption with Bioruptor®

Soluble protein release correlates with cell disruption

Specific enzymatic activity remains after sonication

Sheared DNA is released during bacteria sonication